Academic Journal of Cancer Research 2 (2): 68-72, 2009
ISSN 1995-8943
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Activities of Antioxidant Enzyme and Lipid Peroxidation in Ovarian Cancer Patients

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Abstract: Ovarian cancer is the leading cause of death due to gynecological malignancies among women. The extent of free radical induced oxidative stress can be exacerbated by the decreased efficiency of antioxidant mechanisms. The present study was conducted to investigate the extent of oxidative stress and the levels of antioxidants in the circulation of ovarian cancer patients. Methods: Plasma thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CD) and the levels of antioxidants such as superoxide dismutase (SOD), catalase (CAT), vitamin C and vitamin E were estimated in the circulation of 46 ovarian cancer patients and an equal number of age-matched normal subjects as control. Results: Significantly increased concentrations of plasma TBARS and CD and significantly lowered levels of SOD, CAT, vitamin C and vitamin E were observed in ovarian cancer patients as compared with normal subjects. Conclusion: The low levels of SOD, CAT, vitamin C and vitamin E in the plasma of ovarian cancer patients may be due to their increased utilization to scavenge lipid peroxides as well as their sequestration by tumor cells. Increased levels of lipid peroxidation may be due to excessive oxidative stress caused by incessant ovulation or epithelial inflammation.

Key words: Antioxidants · Free radicals · Ovarian cancer · Oxidative stress · Lipid peroxides

INTRODUCTION

Ovarian cancer is the most lethal gynecologic malignancy with epithelial ovarian neoplasms comprising ovarian tumors in adult women. Approximately two women with epithelial ovarian cancer are diagnosed with advanced-stage disease, contributing to a poor overall survival [1]. Epithelial ovarian neoplasms sub classified histologically into serous, mucinous, endometrioid, clear cell, transitional (Brenner), squamous and undifferentiated subtypes. Serous carcinomas (SC) most common histology, accounting for about thirds of ovarian carcinomas [2].

Endometrioid ovarian carcinoma (EC) is the next most common subtype representing 15% of cases [2]. Both EC and clear carcinomas (CC) may arise in the context of ovarian endometriosis, although the behavior of CC is aggressive [3,4]. Clinically, these subtypes have differences prognosis and response to chemotherapy and expression array analyses indicate that they also distinct gene expression profiles [5,6]. Understanding molecular basis of solid tumors is increasingly important understanding and predicting responses to targeted biological therapeutic agents.

In India, 15% of all gynecological cancers is ovarian malignancy [7] and it represents the greatest clinical challenge. Risk factors for ovarian carcinoma include inflammation, excessive number of life time ovulations, increases in steroid hormone levels, heredity, infertility, oral contraceptive pills, age, asbestos, talc and reproductive factors such as nulliparity [8, 9]. Ovarian cancer at an early stage is asymptomatic, but later the main symptoms include abdominal swelling, bloating, pain and pressure [10]. Recent molecular studies have shown that ovarian cancer has acquired genetic alterations of oncogenes and tumor suppressor genes such as BRCA1, p53, nm23 and K-ras, which may be due to inflammation and oxidative stress [11]. Oxidative stress caused by increased free radical generation and/or decreased antioxidant level in the target cells and tissues has been

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suggested to play an important role in carcinogenesis [12-14] Free radicals are capable of altering all major classes of biomolecules, such as lipids, nucleic acids and proteins, with changes in their structure and function [15]. Prime targets of free radicals are the polyunsaturated fatty acids in cell membranes and their interaction results in lipid peroxidation. The levels of free radical molecules are controlled by various cellular defense mechanisms, consisting of enzymatic catalase, glutathione peroxidase, superoxide dismutase) and non-enzymatic (vit. E, vit. C and glutathione) components [16].

Up to recent times, in studies carried out in order to investigate the role of antioxidants in cancer, they were measured separately; but owing to the various interactions between antioxidants, measuring the total effects of antioxidants in an organism can provide more accurate results [17]. The present study aimed to determine the extent of oxidative stress by measuring TBARS, CD and antioxidant parameters in plasma of the patients with malignant ovarian carcinoma.

Subjects: Forty six newly diagnosed Ovarian cancer patients who had not undergone any previous treatment for their tumors were selected for the study. The mean age of the patients was 52.10 ± 6.93 yr, with the range of 35 to 65 yr were clinically categorized as stage II and III. All the patients and control subjects were non smokers; none of them had concomitant diseases such as diabetes mellitus, liver disease and rheumatoid arthritis. There were 30 healthy volunteers (all women, 50.78±7.89 yr, 35-65 yr old) serving as control subjects. They were from the same socio economic status as that of the cervical cancer patients. Oral consent was obtained from both the cervical patients and the normal subjects before the study. The Human Ethics Committee, India approved the study.

Sample Collection: Blood was collected by venous arm puncture in patients and controls by venous arm punctures into EDTA tubes and the plasma separated by centrifuging at 1000 g for 15 min.

Biochemical Estimations: Lipid peroxidation was estimated by measurement of thiobarbituric acid reactive substances (TBARS) in plasma by the method of Yagi [18]. The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated at 532 nm.

Conjugated dienes were estimated by the method of Rao and Recknagel [19]. This method is based on the arrangement of the double bonds in polyunsaturated fatty acids (PUFA) to form conjugated dienes with an absorbance maximum at 233 nm.

Superoxide dismutase (SOD) was assayed by the method of Kakkar *et al*. [20] based on the 50% inhibition of the formation of NADH-phenazine methosulfate-nitroblue tetrazolium formazan at 520 nm.

Catalase (CAT,) activity was assayed by the method of Sinha [21]. Hemolysate was treated with H_2O_2 (0.2 mol/l) and the reaction was arrested after 60 by the addition of dichromate–acetic acid reagent, cooled and the intensity of color read at 620 nm. Various aliquots of H_2O_2 were used as the standard. A system devoid of the enzyme served as the control.

Plasma vitamin C (ascorbic acid) was estimated by the method of Roe and Kuether [22] in which dehydro ascorbic acid is coupled with 2, 4 dinitro phenyl hydrazine (DNPH) and when treated with sulfuric acid, forms an orange red Color compound, which was measured at 520 nm.

Plasma vitamin E (α - tocopherol) was estimated by the method of Barker and Frank [23]. The method involves the reduction of ferric ions to ferrous ions by α -tocopherol and the formation of a colored complex with 2, 2_ - dipyridyl. Absorbance of the chromophore was measured at 520 nm. Hemoglobin in the hemolysate was measured according to the method of Drabkin and Austin [24]. Blood was diluted in an alkaline medium containing potassium cyanide and potassium ferricyanide. Hemoglobin oxidized to methemoglobin combines with cyanide to form cyanmethemoglobin which was measured at 540 nm The data for biochemical analyses are expressed as mean S.D. Statistical comparisons were performed by Student's t-test using the Statistical Package for Social Sciences version 17.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Table 1 shows the level of lipid peroxidation in plasma of normal and ovarian cancer patients. Lipid peroxidation as assessed by TBARS level was significantly higher in ovarian cancer patients as compared with normal subjects. Plasma conjugated dienes were also significantly increased in ovarian cancer patients as compared with normal subjects. Table 2 shows the level of antioxidants in the circulation of normal and ovarian cancer patients. The enzymatic antioxidants such as SOD and CAT in the hemolysate were significantly lower in ovarian cancer patients as compared with normal subjects. Also the non-enzymatic antioxidants vitamins C and E in the plasma were significantly lower in ovarian cancer patients vs. controls.

Table 1: Lipid peroxidation in ovarian cancer patients (mean ± S.D n = 46)

Parameters	Control	Ovarian Cancer
TBARS (nmol of MDA formed/ml of plasma)	2.11 ± 0.15	5.0 ± 0.35*
CD (imol/ml of plasma)	0.64 ± 0.078	1.63 ± 0.140*

*As compared with ovarian cancer controls, P < 0.001

Table 2: Antioxidant status in ovarian cancer patients (mean ± S.D n = 46)

Parameters	Control	Ovarian Cancer
SOD ([a]U/mg Hb)	1.90 ± 0.27	0.75 ± 0.17*
CAT ([b]U/mg Hb)	5.22 ± 0.29	4.05 ± 0.35*
VITAMINE E (mg/dl of plasma)	2.80 ± 0.23	1.33 ± 0.13*
VITAMINE C (mg/dl of plasma)	1.04± 0.14	0.35 ± 0.13*

a Enzyme concentration required to inhibit the chromogen produced by 50% in 1 min under standard conditions.

b Micromoles of H_2O_2 decomposed.

*P < 0.001 as compared with normal.

DISCUSSION

Oxidative stress is due to a disturbance in the balance between the production of ROS and the efficiency of the antioxidant defense. In other words, oxidative stress results if excessive production of ROS overwhelms the antioxidant defense system or when there is a significant decrease or lack of antioxidant defense [25]. Potential biological targets for free radical attack include lipids, proteins and nucleic acids [26]. The epoxides generated due to increased oxidative stress may spontaneously react with nucleophilic centers in the cell and thereby covalently bind to DNA, RNA and protein. Such reactions may lead to cytotoxicity and carcinogenicity depending on the properties of the epoxides [27].

Moreover, severe oxidative stress is not only known to cause DNA damage and mutations of tumor suppressor genes, which are initial events in carcinogenesis [25], but can also play an important role in the promotion of multistep carcinogenesis [28]. Lipids, especially polyunsaturated fatty acids (PUFA), are very susceptible to free radical attack, which can initiate lipid peroxidation [29]. Lipid peroxidation plays an important role in the control of cell division [30]. The end product of lipid peroxidation, malondialdehyde, due to its high cytotoxicity and inhibitory action on protective enzymes, is suggested to act as a tumor promoter and a co-carcinogenic agent [31]. An inverse relationship has been observed between lipid peroxidation and the rate of cell proliferation, with highly proliferatingtumors showing low levels of lipid peroxidation [32].

Studies show that in contrast todecreased lipid peroxidation in tumor tissues, enhanced lipid peroxidation is observed inthe circulation of cancer patients. In our earlier studies, we have observed increased lipid peroxidation and decreased antioxidant levels in the plasma of cervical cancer patients [33]. In our present work, we noticed increased levels of circulating TBARS and conjugated dienes in ovarian cancer patients which can be attributed to the deficiency of antioxidant defense mechanisms.

The antioxidant enzymes, SOD and CAT, widely distributed in all cells, are present in high amounts in erythrocytes [34]. SOD protects cells against O_2^- by dismutation of the highly reactive superoxide anion to O_2 and to a less reactive species, H_2O_2 [35]. CAT, in turn, protects the cell from H_2O_2 generated by various reactions [36]. In our studies, we observed low levels of SOD and CAT in ovarian cancer patients. The observed increase in circulating lipid peroxides of ovarian cancer patients correlate with the decline in SOD and CAT activity. This can result in accumulation of superoxide anion, highly diffusible and potent oxidizing radical capable of traversing membranes, causing deleterious effects at sites far from the tumor [37].

A decrease in the activity of CAT could be due to increase in the lipid peroxidation product, malondialdehyde which can form cross links, thereby inactivating several membrane bound enzymes [38, 39]. The increase in circulating lipid peroxides may be related to a deficiency of SOD in tumor tissues. Decreased CAT activity could also be due to exhaustion of the enzyme because of increased peroxidation. Vitamin E is a lipid-soluble, chain terminator antioxidant present along with lipids in the cell membranes [40]. Vitamin C, a water-soluble, radical scavenging antioxidant [41], present in all cells can also act as a reducing agent. Vitamin C can neutralize vitamin E radical getting itself converted to a free radical (unreactive) while regenerating vitamin E [42].

A positive correlation between vitamins E and C deficiency and lipid peroxide formation has been documented [43]. In addition to its antioxidant potential, vitamin E also functions as a biologic response modifier influencing the production of second messengers and products of the arachidonic acid cascade which have profound effects on cell proliferation [44]. Epidemiology studies support an inverse relationship between circulating levels of vitamins C and E with ovarian cancer [45, 46]. Due to low economic status, women were malnourished and thus were more prone to malignancy. Studies indicate that women who consume fewer amounts

of fruits and vegetables are associated with ovarian cancer [47]. Thus the enhanced lipid peroxidation observed in ovarian cancer patients can also be attributed to a large extent to the depletion of vitamins E and C in the diet. In our studies also we observed low levels of vitamins C and E in the circulation of ovarian cancer patients. The decreased levels of plasma vitamins E and C may be due to their increased utilization in scavenging lipid peroxides as well as sequestration by tumor cells [33].

Thus, low levels of SOD, CAT, vitamins C and E in the ovarian cancer patients may be due to increased utilization to scavenge lipid peroxides as well as their sequestration by tumor cells. Increased levels of lipid peroxidation may be due to excessive oxidative stress caused by incessant ovulation or epithelial inflammation initiators such as talc and asbestos.

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